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# Phospholipase C in *Dictyostelium discoideum*

## Identification of stimulatory and inhibitory surface receptors and G-proteins

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A combined biochemical and genetic approach was used to show that phospholipase C in the cellular slime mould *Dictyostelium* is under dual regulation by the chemoattractant cyclic AMP (cAMP). This dual regulation involves stimulatory and inhibitory surface receptors and G-proteins. In wild-type cells both cAMP and guanosine 5′-[γ-thio]triphosphate (GTP[S]) stimulated phospholipase C. In contrast, mutant *fgd* A, lacking the G-protein α-subunit Gα2, showed no stimulation by either cAMP or GTP[S], indicating that Gα2 is the stimulatory G-protein. In mutant *fgd* C cAMP did not stimulate phospholipase C, but stimulation by GTP[S] was normal, suggesting that the defect in this mutant is upstream of the stimulatory Gα2. Inhibition of phospholipase C was achieved in wild-type cells by the partial antagonist 3′-deoxy-3′-aminoadenosine 3′,5′-phosphate (3′NH-cAMP). This inhibition was no longer observed in transformed cell lines lacking either the surface cAMP receptor cAR1 or the G-protein α-subunit Gα1; in these cells the agonist cAMP still activated phospholipase C. These results indicate that *Dictyostelium* phospholipase C is regulated via a stimulatory and an inhibitory pathway. The inhibitory pathway is composed of the surface receptor cAR1 and the G-protein G1. The stimulatory pathway consists of an unknown cAMP receptor (possibly the *fgd* C gene product) and the G-protein G2.

## INTRODUCTION

In many organisms, signal transduction via Ins(1,4,5)$P_3$ plays a crucial role in $Ca^{2+}$ homoeostasis and related cellular responses ranging from chemotaxis to cell proliferation (for reviews see [1,2]). In the cellular slime mould *Dictyostelium discoideum* the transition from the unicellular vegetative stage to the developmental multicellular stage is mediated by chemotaxis towards extracellular cyclic AMP (cAMP) [3]. After starvation, cells develop a cAMP-signal-transducing system [4,5]. In response to cAMP, multiple second-messenger systems are activated, including adenylate and guanylate cyclase [5–9]. Apart from these two enzymes generating cAMP and cyclic GMP, *Dictyostelium* also shows an increase in cellular levels of Ins(1,4,5)$P_3$ in response to the chemoattractant cAMP [10–13].

Mutants of the frigid class (*fgd*) are defective in cAMP-induced development [14]. Previous work showed that Ins(1,4,5)$P_3$ signalling is impaired in mutants *fgd* A and *fgd* C [15–17]. In the mutant *fgd* A nearly all signal transduction is blocked, due to a deletion in one of the α-subunits of G-proteins, Gα2 [15,18]. The primary defect in the mutant *fgd* C is still unknown. This mutant shows relatively normal cAMP and cyclic GMP responses but no increase in Ins(1,4,5)$P_3$ levels after stimulation of cells with cAMP. At the same time this mutant needs 100-fold higher concentrations of cAMP for a chemotactic response as compared with wild-type cells [16].

By using analogues of cAMP, it was shown not only that Ins(1,4,5)$P_3$ levels may increase upon stimulation, but that a decrease of Ins(1,4,5)$P_3$ levels is also possible [19]. Analogues which were shown previously to act as partial antagonists [20] induced inhibition of Ins(1,4,5)$P_3$ production *in vivo*. This inhibition of Ins(1,4,5)$P_3$ production correlates well with the observed antagonism for chemotaxis. The data described above indicate that, apart from a simulatory pathway, phospholipase C activity in *Dictyostelium* is regulated by an inhibitory pathway as well. Recently several reports suggest such a dual regulation of phospholipase C in other organisms [21–27].

To date, four different genes coding for cAMP surface receptors and eight different genes coding for G-protein α-subunits have been cloned in *Dictyostelium* [28–32]. By using homologous recombination, cell lines were made lacking the surface receptor cAR1 or the G-protein α-subunits Gα1, Gα2 or Gα3 [33,34]; M. Pupillo and P. N. Devreotes, unpublished work). These transformed cells, together with signal-transduction mutants and cAMP antagonists, provide a powerful tool for investigating the function of receptors and G-proteins in specific parts of the transmembrane signal-transduction pathways. In the preceding paper [35] a receptor- and G-protein-sensitive phospholipase C activity in *Dictyostelium discoideum* was characterized. Here we used a combined approach of mutants, transformants and cAMP analogues to identify the receptors and G-proteins involved in the dual regulation of phospholipase C activity in *Dictyostelium*.

## MATERIALS AND METHODS

### Chemicals

[$^3$H]Ins(1,4,5)$P_3$ (20–60 μCi/mmol) was obtained from Amersham International. Guanosine 5′-[α-thio]triphosphate (GTP[S]) was obtained from Boehringer Mannheim. cAMP, EGTA and Hepes were from Sigma. 3′-Deoxy-3′-aminoadenosine 3′,5′-monophosphate (3′-NH-cAMP) was kindly given by Dr. B. Jastorff (University of Bremen, Germany); all other chemicals used were of at least analytical grade.

### Cells and cell culture

Cell strains AX3, JH130, JH131, HPS400, G3T2, 1A3 and ⌂280 were grown in axenic medium HG-5, which is an adapted form of the HL-5 medium [36] containing 10 g/l instead of 16 g/l

Abbreviations used: cAMP, cyclic AMP; GTP[S], guanosine 5′-[γ-thio]triphosphate; 3′NH-cAMP, 3′-deoxy-3′-aminoadenosine 3′,5′-monophosphate.

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glucose. Cell strains NC-4, HC-85, HC-317, XP-55 and HC-6 were grown in co-culture with *Klebsiella aerogenes* on solid medium containing 3.3 g/l glucose, 3.3 g/l peptone and 10 mM Na/K phosphate buffer, pH 6.5. Axenically grown strains were harvested in the late-exponential phase, and bacterial-grown strains just before clearing of the bacterial lawn. Cells were starved for 4 h by shaking in Na/K phosphate buffer, pH 6.5, to acquire aggregation competence.

### Ins(1,4,5)$P_3$ production *in vivo*

Aggregation-competent cells were harvested, washed once in 40 mM Hepes/NaOH, pH 6.5, and resuspended in this buffer at $5 \times 10^7$ cells/ml. Before starting the experiment, cells were aerated for 10 min, and aeration was continued during the experiment. Stimulation of cells was performed by adding 270 $\mu$l of cell suspension to 30 $\mu$l of 10-times-concentrated stimulus. Subsequently 50 $\mu$l samples were withdrawn at the indicated times and quenched by addition of an equal volume of 3.5 % $HClO_4$. The zero-time points were obtained by taking 45 $\mu$l of cell suspension and adding this to 50 $\mu$l of 3.5 % $HClO_4$ plus 5 $\mu$l of stimulus just before the stimulation of the other cells.

### Phospholipase C activity *in vitro*

Phospholipase C activity was determined as described in the preceding paper [35]. In short, cells were harvested and resuspended as described above. Portions of cells were stimulated with cAMP and lysed in the presence of 5.9 mM EDTA; 10 s after lysis, part of the sample was quenched with an equal volume of 3.5 % $HClO_4$ and the other part was adjusted to 10 $\mu$M free $Ca^{2+}$ by addition of 5.9 mM $CaCl_2$ and incubated for another 20 s before being quenched. Ins(1,4,5)$P_3$ levels were determined in samples neutralized with $KHCO_3$, by using an isotope-dilution assay as described previously [13,37]. Phospholipase C activity is defined as the amount of Ins(1,4,5)$P_3$ produced during the 20 s incubation with $Ca^{2+}$.

Ins(1,4,5)$P_3$ phosphatase activity was measured as described in the preceding manuscript [35].

## RESULTS

### Mutants *fgd* A and *fgd* C defective in phospholipase C stimulation

Stimulation of *fgd* C and *fgd* A cells with the chemoattractant cAMP *in vivo* does not elicit an increase in Ins(1,4,5)$P_3$ levels; in fact, cAMP induces a small decrease in both strains (Figure 1a). To investigate the nature of the defect in these mutants, the activation of phospholipase C by cAMP and GTP[S] was investigated *in vitro*. Mutant cells were stimulated with cAMP and phospholipase C activity was determined. Basal phospholipase C activity in these mutants is not significantly different from the activity of wild-type cells. Whereas cAMP stimulates phospholipase C activity about 2-fold in wild-type cells, addition of cAMP to these mutants leads to the inhibition of basal phospholipase C activity (Figure 1b); activities relative to unstimulated activity are: wild-type 216 ± 63 %, *fgd* A 71 ± 37 %, *fgd* C 42 ± 20 %.

The non-hydrolysable GTP analogue GTP[S] was used to activate G-proteins. In wild-type cells GTP[S] stimulates phospholipase C about 2-fold [35]. Figure 1(b) demonstrates that GTP[S] does not stimulate phospholipase C in *fgd* A cells, suggesting that the G$\alpha$2 protein, which is impaired in *fgd* A, is responsible for activation of phospholipase C. In contrast, *fgd* C shows a wild-type response towards GTP[S] *in vitro*, indicating that G-protein/phospholipase C coupling is intact in this mutant (Figure 1b). Since receptor-stimulated activity is lost, this suggests that the defect in *fgd* C is upstream from the G-protein. All current models for G-protein-based signal transduction have only one component upstream of the G-protein, i.e. the receptor; *fgd* C may be a stimulatory receptor mutant.

Several observations, including the inhibition of phospholipase C by cAMP in *fgd* A and *fgd* C (Figure 1), suggest the presence of another receptor- and G-protein-based mechanism involved in the decrease of phospholipase C activity.

### Inhibition of phospholipase C by the cAMP analogue 3′NH-cAMP

The hypothesis for a G-protein-coupled inhibitory pathway of phospholipase C is supported by the finding that certain cAMP

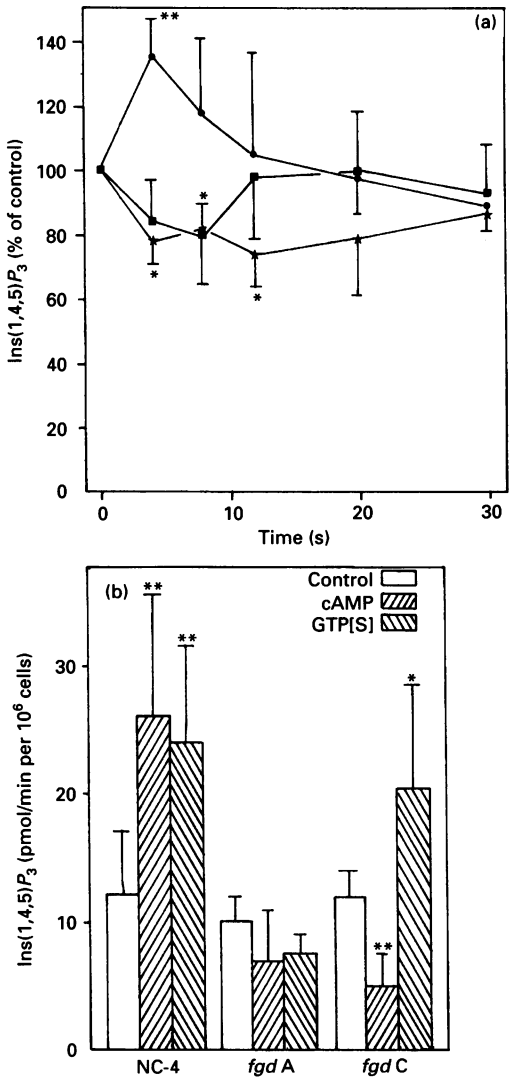


**Figure 1 Ins(1,4,5)$P_3$ levels and phospholipase C activity in *fgd* A and *fgd* C mutant strains**

(**a**) Ins(1,4,5)$P_3$ response after stimulation of cells with 0.1 $\mu$M cAMP: ●, wild-type cells; ★, *fgd* A strain HC85; ■, *fgd* C strain HC 317. Data are presented as means ± S.E.M. of 4 independent experiments in triplicate. (**b**) Phospholipase C activity *in vitro* after stimulation of cells *in vivo* with 1 $\mu$M cAMP or *in vitro* with 10 $\mu$M GTP[S]. Data are expressed as means ± S.E.M. of 3 independent experiments in duplicate: significant differences from control are shown by $^*P < 0.05$ and $^{**}P < 0.01$.

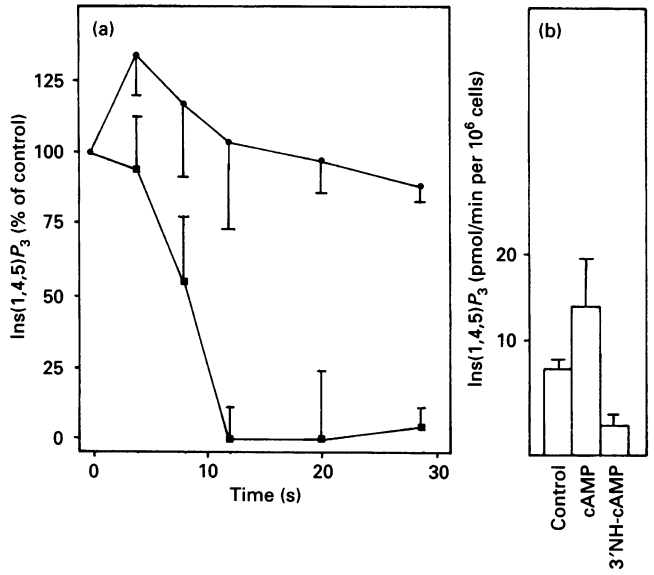


**Figure 2 Effect of the partial antagonist 3'NH-cAMP on Ins(1,4,5)$P_3$ levels *in vivo* and phospholipase C activity *in vitro***

(**a**) Time course of Ins(1,4,5)$P_3$ levels after stimulation of wild-type cells with 1 $\mu$M cAMP (●) or 10 $\mu$M 3'NH-cAMP (■). Data are expressed as means ± S.E.M. of 3 independent experiments in triplicate. (**b**) Phospholipase C activity *in vitro* after stimulation of cells *in vivo* with 1 $\mu$M cAMP or with 10 $\mu$M 3'NH-cAMP.

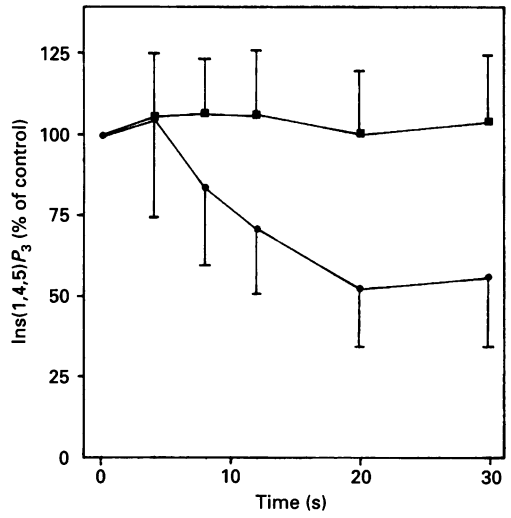


**Figure 3 Effect of the partial antagonist 3'NH-cAMP on Ins(1,4,5)$P_3$ levels in the Gα1-null cell line JH131**

Cells were stimulated with 10 $\mu$M 3'NH-cAMP, and Ins(1,4,5)$P_3$ levels were determined. ●, Control cells (JH130); ■, Gα1-null cells (JH131). Data are expressed as means ± S.E.M. of 3 independent experiments in triplicate.

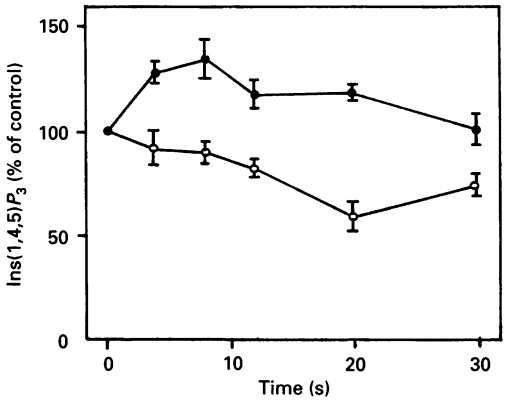


**Figure 4 Effect of the partial antagonist 3'NH-cAMP on Ins(1,4,5)$P_3$ levels in the cAR1-null cell line ⌂280**

Cells were stimulated with 10 $\mu$M 3'NH-cAMP, and Ins(1,4,5)$P_3$ levels were determined: ○, control cells (1A3); ■, cAR1-null cells (⌂280). Data are means ± S.E.M. of 3 independent experiments in triplicate.

analogues such as 3'NH-cAMP induce a decrease in Ins(1,4,5)$P_3$ levels *in vivo*. In Figure 2 the effect of 3'NH-cAMP both on Ins(1,4,5)$P_3$ levels *in vivo* as well as on phospholipase C activity *in vitro* is shown. *In vivo* the analogue induces a rapid decrease in Ins(1,4,5)$P_3$ levels, with hardly any Ins(1,4,5)$P_3$ remaining at 30 s after stimulation (Figure 2a). *In vitro* a strong inhibition of phospholipase C activity is observed after stimulation with the analogue. Basal phospholipase C activity is decreased to 30% of that in untreated cells (Figure 2b).

Apparently 3'NH-cAMP activates an inhibitory pathway of phospholipase C. Although no reports are available to date on G-protein-coupled stimulation of Ins(1,4,5)$P_3$ phosphatases, this possibility could not be excluded as a partial source for the observed decrease in Ins(1,4,5)$P_3$ *in vivo*. Therefore Ins(1,4,5)$P_3$ phosphatase activity after stimulation of cells with 3'NH-cAMP was measured. No difference in Ins(1,4,5)$P_3$ phosphatase activity in lysates of untreated cells compared with 3'NH-cAMP-treated cells was observed (results not shown).

## Identification of the receptors and G-proteins involved in phospholipase C inhibition

Since 3'NH-cAMP was recognized as a selective activator of the inhibitory pathway, it was used to identify the receptor and G-protein that mediate inhibition of phospholipase C activity. Cell lines with a gene disruption of one of the possible components were tested for activation and inhibition of phospholipase C in response to cAMP or the analogue 3'NH-cAMP. To date, eight different Gα subunits have been cloned, and four different cAMP receptors [28–32]. By using homologous recombination, cell lines have been created carrying three different Gα-gene disruptions, i.e. Gα1, Gα2 (=*fgd* A) and Gα3, and a receptor-gene disruption for cAR1 ([32,33]; M. Pupillo and P. N. Devreotes, unpublished work).

Cells with a disruption of the Gα3 gene show a normal decrease in Ins(1,4,5)$P_3$ levels in response to 3'NH-cAMP (results not shown), indicating that Gα3 does not mediate inhibition of phospholipase C. The Gα2-null cell line was not tested with 3'-NH-cAMP, since this cell line is comparable with the *fgd* A strain HC-85, which revealed that Gα2 is the stimulatory G-protein. Figure 3 shows the effect of 3'NH-cAMP on the Ins(1,4,5)$P_3$ levels in the Gα1-null strain and its isogenic control cell line, JH131 and JH130 respectively. Whereas 3'NH-cAMP induces a decrease in Ins(1,4,5)$P_3$ levels in control cells, the Gα1-null cells shows neither a decrease in the cellular Ins(1,4,5)$P_3$ level *in vivo* nor a decrease in phospholipase C activity *in vitro* in response to 3'NH-cAMP (Figure 3). Thus in the Gα1-null cells the inhibitory pathway to phospholipase C has been disrupted, which identifies

Gα1 as the G-protein coupling the receptor for 3′NH-cAMP to inhibition of phospholipase C.

In order to find the function of the surface cAMP receptor cAR1 in the regulation of phospholipase C, cAR1-null cells were stimulated with 3′NH-cAMP and cAMP. The results show that disruption of cAR1 leads to the absence of 3′NH-cAMP-induced inhibition of phospholipase C (Figure 4), whereas cAMP still stimulates the enzyme (results not shown). Thus the receptor coupling the inhibitory action of 3′-NH-cAMP to phospholipase C is coded for by cAR1. Since cAMP stimulation of phospholipase C is unimpaired in these cells, cAR1 appears not to be essential in the stimulatory pathway.

## DISCUSSION

Regulation of phospholipase C has been studied extensively in many organisms and cell types, and several reports on the inhibition of phospholipase C are known. Basically two mechanisms are proposed for this inhibition: on the one hand, phosphorylation of activating compounds through the action of either protein kinase C, providing a negative-feedback mechanism, or protein kinase A, allowing cross-talk between cAMP and Ins(1,4,5)$P_3$ signalling [21–24]; on the one hand, a direct inhibitory action of G-proteins. The evidence for this latter possibility is based on indirect observations, e.g. the effect of guanine nucleotides and NaF on the activation of phospholipase C [25–27]. In the present paper we provide further genetic evidence for the presence of a receptor/G-protein-mediated inhibitory pathway for phospholipase C and identify the receptor and G-protein involved.

Using a combined approach of pharmacological and molecular-genetic techniques to elucidate the regulatory components involved in the modulation of phospholipase C activity in *Dictyostelium discoideum*, two regulatory pathways can be discerned, a stimulatory pathway activated by cAMP and an inhibitory one which is specifically activated by the analogue 3′NH-cAMP. This feature of 3′-NH-cAMP allows pharmacological separation of the two different pathways. It is likely that cAMP activates both the stimulatory and the inhibitory pathway, since the cAMP-dose–response curve of phospholipase C stimulation is bell-shaped, with stimulation at low cAMP concentrations and inhibition at high concentrations [35]. Using cell lines with deletions of either specific G-protein α-subunits or cAR1, the components involved in phospholipase C regulation were identified; the results are summarized in Table 1. cAR1 was identified as the inhibitory receptor and Gα1 as the inhibitory G-protein α-subunit, since removal of either of these two proteins through homogolous recombination resulted in the loss of 3′NH-cAMP-mediated inhibition of phospholipase C. The stimulatory pathway is composed of an unknown receptor and Gα2 as the α-subunit of the stimulatory G-protein. The latter was shown by the lack of phospholipase C activation in mutant *fgd* A by either cAMP or GTP[S]. The identity of the stimulatory receptor remains to be established. To date, four different cAMP receptors have been identified genetically in *Dictyostelium*, designated cAR1–4. As shown above, cAR1 is the inhibitory receptor, and cAR2 and cAR4 are not expressed in aggregation-competent cells, which would leave only cAR3 as a possible candidate. However, the binding specificity of cAR1-3 for various cAMP analogues is very similar, including the binding specificity for 3′NH-cAMP [38]. This implies that cAR3 would bind 3′NH-cAMP as efficiently as cAR1. Since the stimulatory receptor is not effected by 3′NH-cAMP, involvement of cAR3 is unlikely, which would mean that the stimulatory receptor is an unknown cAR. It is possible that the stimulatory receptor is the product of the *fgd* C gene, which is suggested by the observation that the defect in *fgd* C is upstream of Gα2. Final proof of this hypothesis can only be obtained by cloning the *fgd* C gene. Restoration of stimulation of phospholipase C could be used in complementation assays.

**Table 1 Summary of the regulation of phospholipase C in *Dictyostelium***

Key: +, stimulation; −, inhibition; 0, no effect; 0/−, slight inhibition; ND, not determined.

| Cells | Defective protein | Phospholipase C after stimulation with | | |
|---|---|---|---|---|
| | | cAMP | 3′NH-cAMP | GTP[S] |
| Wild-type | none | + | − | + |
| *fgd* A | Gα2 | 0/− | − | 0/− |
| *fgd* C | ? | 0/− | − | + |
| JH131 | Gα1 | + | 0 | + |
| G3T2 | Gα3 | + | − | + |
| Δ280 | cAR1 | + | 0 | ND |

Thus a model for the regulation of phospholipase C in *Dictyostelium* emerges. Figure 5 is a schematic representation of this model, in which all components, with the exception of the stimulatory receptor, have been characterized and their respective genes cloned, including phospholipase C [39]. During development of wild-type cells phospholipase C is inhibited by GTP[S] during growth and stimulated during cell aggregation [35]. This differential regulation coincides with the expression of the regulatory G-proteins. The inhibitory Gα1 is expressed throughout development, whereas the stimulatory Gα2 only during cell aggregation. The interaction of two different G-proteins with phospholipase C, the simple and fast assay for phospholipase C and the possibility to create cell lines lacking either of these G-proteins provides a powerful tool to study the interaction between G-proteins and phospholipase C. Using chimeras of G1 and G2, the functional domains of G-proteins interacting with phospholipase C can be determined.

In most eukaryotic cells one hormone activates multiple effector enzymes. Specificity of signal transduction is generally provided by the effector enzyme being activated by a specific G-protein and sometimes by a specific receptor. The current model for signal transduction in *Dictyostelium* is largely based on the observations made with *fgd* A. This mutant lacks one G-protein and is defective in nearly all sensory transduction *in vivo*, including the activation of adenylate cyclase, guanylate cyclase and phospholipase C [14,15,17]. Since GTP[S] stimulation of adenylate and guanylate cyclases *in vitro* are essentially normal, and GTP[S] stimulation of phospholipase C is absent, a hierarchy of signal transduction was proposed, with G2 and its effector (possibly phospholipase C) at the heart and adenylate and guanylate cyclases downstream. The present results are probably not in accordance with such a model. The defect of mutant *fgd* C for the activation of phospholipase C is upstream of G2. Whereas the *fgd* A gene product Gα2 is essential for nearly all signal transduction, the *fgd* C gene product is not required for the activation of adenylate and guanylate cyclases [14,16]. These considerations suggest several parallel signal-transduction routes in *Dictyostelium*, in which G2 is essential for the coupling of the stimulatory receptors to the different effectors. Specificity of signal transduction may be provided by three non-exclusive mechanisms: (i) different receptors, (ii) other G-proteins, and (iii) other intracellular proteins. Arguments for each of these models are available. Receptor: if the *fgd* C gene product is a receptor, as tentatively suggested in the present paper, then this

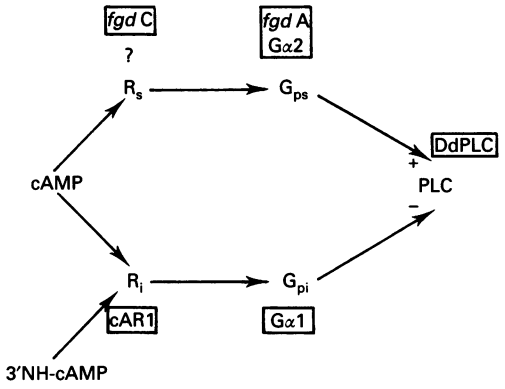


**Figure 5 Model for the regulation of phospholipase C**

Mutants and known genes are indicated by boxes. DdPLC is the *Dictyostelium* PLC gene [39]. The identities of the stimulatory receptor $R_s$ and *fgd* C are unknown. The *fgd* C gene product functions upstream of the G-protein G2. Abbreviations used: $R_s$, stimulatory receptor; $R_i$, inhibitory receptor; $G_{ps}$, stimulatory G-protein; $G_{pi}$, inhibitory G-protein.

receptor is not involved in the activation of adenylate and guanylate cyclases [16]. G-protein: cAMP-mediated stimulation of adenylate cyclase is absent in G2-null cells; however, GTP[S]-mediated stimulation of this enzyme is normal in membranes, indicating that, besides G2, another G-protein is required for stimulation of adenylate cyclase [15]. Other proteins: different cytosolic proteins are essential for the optimal activity of adenylate cyclase [7,40] and guanylate cyclase [41]; these associated proteins could be involved in the routing of the cAMP signal from one receptor or G-protein to the different effector enzymes.

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## REFERENCES

1 Berridge, M. J. and Irvine, R. F. (1989) Nature (London) **341**, 197–205
2 Rhee, S. G., Suh, P. G., Ryu, S. H. and Lee, S. Y. (1989) Science **244**, 546–550
3 Caterina, M. J. and Devreotes, P. N. (1991) FASEB J. **5**, 3078–3085
4 Devreotes, P. N. and Steck, T. L. (1979) J. Cell Biol. **80**, 300–309
5 Roos, W., Scheidegger, C. and Gerisch, G. (1977) Nature (London) **266**, 259–261
6 Klein, C. (1976) FEBS Lett. **68**, 125–128
7 Theibert, A. and Devreotes, P. N. (1986) J. Biol. Chem. **261**, 15121–15125
8 Mato, J. M., Krens, F. A., Van Haastert, P. J. M. and Konijn, T. M. (1977) Proc. Natl. Acad. Sci. U.S.A. **74**, 2348–2351
9 Janssens, P. M. W., De Jong, C. C. C., Vink, A. A. and Van Haastert, P. J. M. (1989) J. Biol. Chem. **264**, 4329–4335
10 Europe-Finner, G. N. and Newell, P. C. (1987) J. Cell Sci. **87**, 513–518
11 Europe-Finner, G. N., Gammon, B., Wood, C. A. and Newell, P. (1989) J. Cell Sci. **93**, 585–592
12 Van Haastert, P. J. M., De Vries, M. J., Penning, L. C., Roovers, E., Van Der Kaay, J., Erneux, C. and Van Lookeren Campagne, M. M. (1989) Biochem. J. **258**, 577–586
13 Van Haastert, P. J. M. (1989) Anal. Biochem. **177**, 115–119
14 Coukell, M. B., Lappano, S. and Cameron, A. M. (1983) Dev. Genet. **3**, 283–297
15 Kesbeke, F., Snaar-Jagalska, B. E. and Van Haastert, P. J. M. (1988) J. Cell Biol. **107**, 521–528
16 Bominaar, A. A., Kesbeke, F., Snaar-Jagalska, B. E., Peters, D. J. M., Schaap, P. and Van Haastert, P. J. M. (1991) J. Cell Sci. **100**, 825–831
17 Bominaar, A. A., Van der Kaay, J. and Van Haastert, P. J. M. (1991) Dev. Gen. **12**, 19–24
18 Kumagai, A., Pupillo, M., Gundersen, R., Maike-Lye, R., Devreotes, P. N. and Firtel, R. A. (1989) Cell **57**, 265–275
19 Peters, D. J. M., Bominaar, A. A., Snaar-Jagalska, B. E., Brandt, R., Van Haastert, P. J. M., Ceccarelli, A., Williams, J. G. and Schaap, P. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 9212–9223
Van Haastert, P. J. M. (1983) J. Biol. Chem. **258**, 9643–9648
Connolly, T. M., Lawing, W. J. J. and Majerus, P. W. (1986) Cell **46**, 951–958
Kato, H., Ishitoya, J. and Takenawa, T. (1986) Biochem. Biophys. Res. Commun. **139**, 1272–1278
23 Sim, S. S., Kim, J. W. and Rhee, S. G. (1990) J. Biol. Chem. **265**, 10367–10372
24 Ryu, S. H., Kim, U. H., Wahl, M. I., Brown, A. B., Carpenter, G., Huang, K. P. and Rhee, S. G. (1990) J. Biol. Chem. **265**, 17941–17945
25 Van Geet, C., Deckmyn, H., Kienast, J., Wittevrongel, C. and Vermylen, J. (1990) J. Biol. Chem. **265**, 7920–7926
26 Bizzarri, C., Di Girolamo, M., D'Orazio, M. C. and Corda, D. (1990) Proc. Natl. Acad. Sci. U.S.A. **87**, 4889–4893
27 Journot, L., Homburger, V., Pantaloni, C., Priam, M., Bockaert, J. and Enjalbert, A. (1987) J. Biol. Chem. **262**, 15106–15110
28 Klein, P., Sun, T. J., Saxe, C. L., Kimmel, A. R., Johnson, R. and Devreotes, P. N. (1988) Science **241**, 1467–1472
29 Saxe, C. L., Klein, P., Sun, T. J., Kimmel, A. R. and Devreotes, P. N. (1988) Dev. Gen. **9**, 227–235
30 Pupillo, M., Kumagai, A., Pitt, G. S., Firtel, R. A. and Devreotes, P. N. (1989) Proc. Natl. Acad. Sci. U.S.A. **86**, 4892–4896
31 Wu, L. and Devreotes, P. N. (1991) Biochem. Biophys. Res. Commun. **179**, 1141–1147
32 Hadwiger, J. A., Wilkie, T. M., Stratmann, M. and Firtel, R. A. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 8213–8217
33 Sun, T. J. and Devreotes, P. N. (1991) Genes Dev. **5**, 572–582
34 Kumagai, A., Hadwiger, J. A., Pupillo, M. and Firtel, R. A. (1990) J. Biol. Chem. **266**, 1220–1228
35 Bominaar, A. A., Kesbeke, F. and Van Haastert, P. J. M. (1994) Biochem. J. **297**, 181–187
36 Coccuci, S. M. and Sussman, M. (1970) J. Cell Biol. **45**, 399–407
37 Donie, F. and Reiser, G. (1989) FEBS Lett. **254**, 155–158
38 Johnson, R. L., Van Haastert, P. J. M., Kimmel, A. R., Saxe, C. L., III, Jastorff, B. and Devreotes, P. N. (1992) J. Biol. Chem. **267**, 4600–4607
39 Drayer, A. L. and Van Haastert, P. J. M. (1992) J. Biol. Chem. **267**, 18387–18392
40 Van Haastert, P. J. M., Snaar-Jagalska, B. E. and Janssens, P. M. W. (1987) Eur. J. Biochem. **162**, 251–258
41 Schulkes, C. C. G. M., Schoen, C. D., Arents, J. and Van Driel, R. (1992) Biochim. Biophys. Acta **1135**, 73–78

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